



## Synthesis of Silver Nanoparticles Using Fermented Solutions of Vegetables, Herbs, and Rhizomes Origin as Bioreductor

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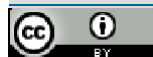
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### ABSTRACT

Silver nanoparticles are becoming a trend in producing products beneficial to humans. They can be made using bioreductor in the form of biological materials, such as ecoenzymes. This study aims to synthesize silver nanoparticles using fermented solutions from purple vegetables, herbs, and spices as bioreductor. This study was conducted in two stages: silver nanoparticle biosynthesis and morphological analysis and antioxidant analysis. The results showed that the silver nanoparticle solution A was successfully synthesized, and solution B had the highest activity value. Therefore, further efforts are needed to explore solution B for further purposes.

## INTRODUCTION

Silver nanoparticles (AgNPs) are nanomaterials known as antimicrobial agents with widespread applications in almost every area of human life. AgNPs can be produced through chemical, physical, or biological processes (Ijaz et al., 2020). Synthesizing AgNPs through biological agents involves plant extracts and other biological materials. One advantage of this synthesis method is its environmental friendliness, as the green AgNP synthesis process does not use hazardous chemical compounds. Several recent studies have shown success in the green synthesis of nanoparticles using bioreductants, for example using yeast (Liu et al., 2021).

Fermentation solutions, such as those made from vegetables, fruit, flowers, or rhizomes, naturally contain various primary and secondary metabolites, such as organic acids, phenolics, and aromatic compounds (Abbaspour, 2024). These active compounds then reduce  $\text{Ag}^+$  ions to  $\text{Ag}^0$  and stabilize the resulting nanoparticles (Sati et al., 2025). Silver nanoparticles with *Muntingia calabura* fruit extract as a bioreductant have been reported to have quite good anticancer, antioxidant, and antibacterial activity (Sivakami et al., 2025). The same thing was reported by Geyesa et al., (2024) in a silver nanoparticle solution using *Verbascum sinaiticum* leaves as bioreductor.

However, until now there has been no report related to the green synthesis of silver nanoparticles from purple vegetables, herbs, and rhizomes as bioreductants so this research is considered necessary. This study aims to synthesize silver nanoparticles with bioreductants in the form of purple vegetables and rhizomes. The results of this study are expected to provide additional scientific information regarding the use of silver nanoparticles from fermentation solutions as solutions that are beneficial for humans, especially as antioxidants.

## METHODOLOGY

### *Making the Fermentation Solution*

The fermentation solution is made by mixing purple eggplant (300 grams), purple cabbage (174 grams), kaffir lime (83 grams), cinnamon (6 grams), cloves (2 grams), galangal (18 grams), lemongrass (38 grams), palm sugar (110 grams), and 2L of water. The mixture is fermented for 1 month, opening the bottle cap daily for the first 7 days, and then weekly thereafter.

### *Biosynthesis of silver nanoparticles*

Silver nanoparticle preparation was carried out according to Nalawati et al., (2021). The fermentation solution was placed in a test tube and 0.01 M silver nitrate solution (Sigma Aldrich) was added in a ratio of 1:5 (A solution; E1P5); 1:10 (B solution; E1P10); and 1:15 (C solution; E1P15). The solutions were then heated to 80°C for 5 minutes and cooled.

### *Morphological and spectrophotometric analysis*

The color of the fermentation solution containing  $\text{AgNO}_3$  solution was observed before and after heating. The absorbance of the resulting color was analyzed in the range of 200–700 nm.

### *Antioxidant analysis*

Antioxidant analysis in this study refers to Gaele Djoueudam et al., (2019). To 100  $\mu\text{L}$  of extract diluted with methanol at several concentrations, 900  $\mu\text{L}$  of DPPH was added, incubated in the dark for 30 minutes, and the absorbance was measured at a wavelength of 517 nm.

## RESEARCH RESULT AND DISCUSSION

### *Morphological and spectrophotometric analysis*

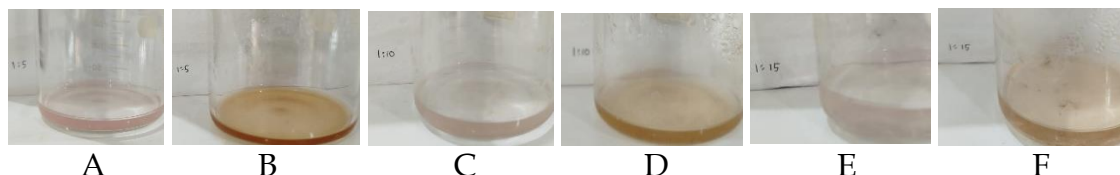
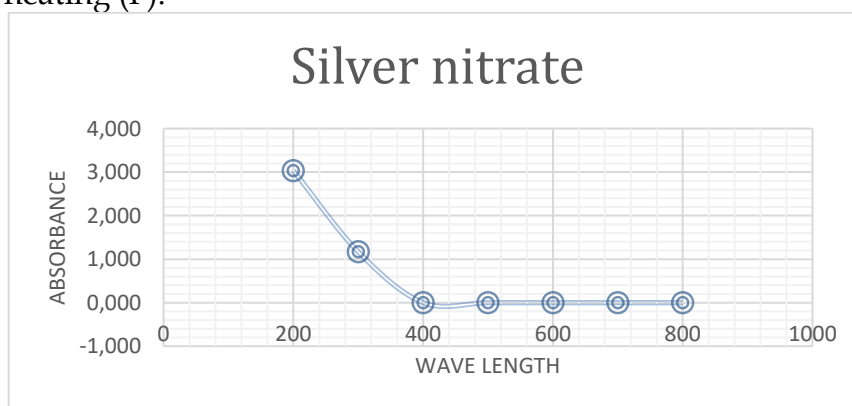
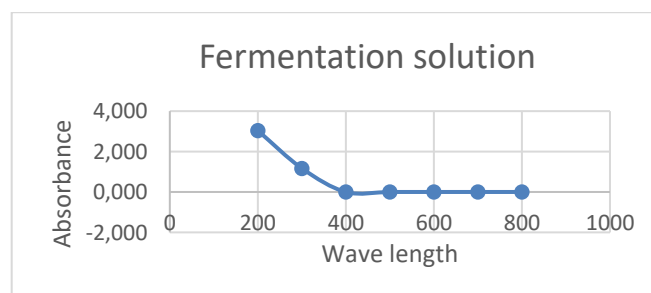


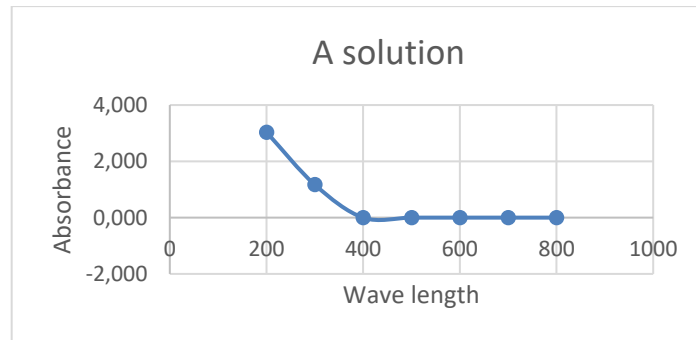
Figure 1. Observation of color changes that formed before to after the heating process in the biosynthesis process of silver nanoparticle. Fermentation solution mixed with silver nitrate in a ratio of 1:5 before heating (A); fermentation solution mixed with silver nitrate in a ratio of 1:5 after heating (B); fermentation solution mixed with silver nitrate in a ratio of 1:10 before heating (C); fermentation solution mixed with silver nitrate in a ratio of 1:10 after heating (D); fermentation solution mixed with silver nitrate in a ratio of 1:15 before heating (E); and fermentation solution mixed with silver nitrate in a ratio of 1:5 after heating (F).



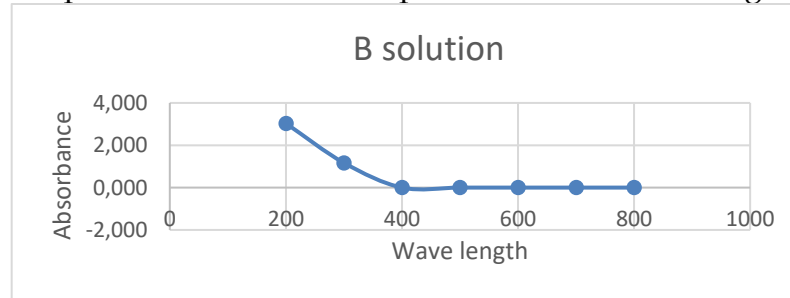
Graphic 1. Silver nitrate absorption at several wavelengths



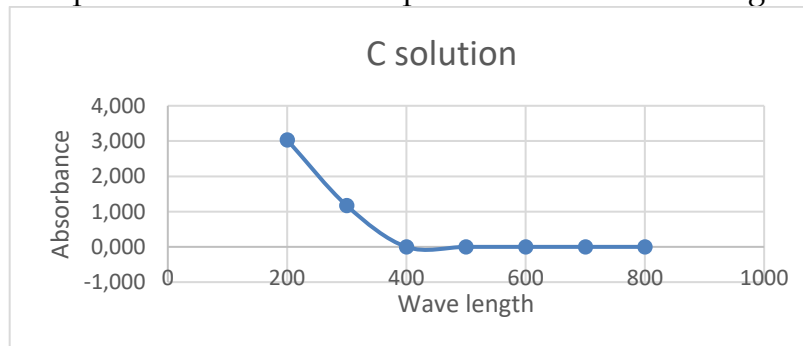
Graphic 2. Fermentation solution absorption at several wavelengths



Graphic 3. A solution absorption at several wavelengths



Graphic 4. B solution absorption at several wavelengths



Graphic 5. C solution absorption at several wavelengths

Morphological observations showed a color change before and after the silver nitrate and fermentation solution were heated (Figure 1). The color change from pink to brown is usually analogous to the formation of silver nanoparticles. This has been widely reported in the biosynthesis of silver nanoparticles using biological materials (Mashau et al.,(2023); Ilahi et al., (2021); Hemlata et al., (2020)).

Spectrophotometer analysis showed a peak, but it was not very significant (Graphic 1-5). This was likely due to the polydispersity of particle size or shape, causing the SPR peak to spread out and appear less steep (Agustina et al., 2021). The influence of bioactive groups in plant extracts can also be the reason for the peaks formed being less significant. This is because plant extracts are complex mixtures of various metabolites (phenolics, proteins, polysaccharides, pigments, etc.) that act as reducing agents, but also attack and cover the surface of the nanoparticles (capping/stabilizer). Furthermore, these compounds can cause different micro-environments on the surface of each particle, modifying their plasmon resonance and these complex interactions often make the SPR peaks very broad/not sharp, due to the variation in the dipole environment around the nanoparticles (Shafie et al., 2021). Interactions between particles can also cause the peaks to be less steep. This is related to nanoparticles adhering to each

other/aggregating, which also affects the shape of the SPR spectrum, so the dark brown color could be a sign of aggregation rather than just particle growth (Azad et al., 2023).

**Table 1. Antioxidant analysis**

No	Solution	Antioxidant activity (IC <sub>50</sub> $\mu\text{g}\cdot\text{mL}^{-1}$ )
1	Fermentation solution	73.69
2	A solution	90.48
3	B solution	57.46
4	C solution	81.48

Antioxidant analysis showed that solution B had the highest antioxidant activity compared to the other two treatments and the fermentation solution without the addition of silver nitrate solution (Table 1). Although all treatments showed strong antioxidant activity, solution B was deemed more promising for further exploration, for example, as an antioxidant in plant tissue culture. ).

Based on this analysis, it can be seen that them all have antioxidant activity with the same strength because they have IC<sub>50</sub> values in the range of 50-100. IC<sub>50</sub> values in this range are classified as solutions that have a "strong" value of antioxidant activity (Itam et al., 2021). This indicated that the addition of silver nitrate solution successfully increased the antioxidant activity of this fermentation solution. The reason of this might be because of the phytochemicals content that present on the surface of the nanoparticles act as stabilizers and provide additional bioactive activity to AgNPs (Alharbi et al., 2022). This is in line with Ilahi et al., (2021) using *Duchesnea indica* root extract and Salayová et al., (2021) using some medicinal plant extract.

## CONCLUSIONS AND RECOMMENDATIONS

The biosynthesis of silver nanoparticles using a fermentation solution containing purple vegetables, herbs, and rhizomes was successfully synthesized. Morphological observations showed a color change to brown and a peak at a wavelength of 300-500 nm. Antioxidant activity tests showed that solution B had the best value. Further research is needed to confirm whether solution B can be explored for further use.

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